United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Developmental toxicity assessments of drinking water contaminants in rats.
LAPR Number:	17-10-001
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6 /RTP/USEPA/US
Date Originated:	08/20/2014
LAPR Expiration Date:	10/31/2017
Agenda Date:	10/22/2014
Date Approved:	10/30/2014
Date Closed:	10/30/2017

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	10/30/2014	Designated Member Reivewer	
	Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	10/30/2014	2nd DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Developmental toxicity assessments of drinking water contaminants in rats.

Is this a continuing study with a previously approved LAPR?
No

2. What is the Intramural Research Protocol (IRP) number covering this project?
IRP: NHEERL-RTP/TAD/ETB/[redacted]/2014-001-r0
Safe and Sustainable Water Resources (SSWR) Task 2.2.D: Integrated Assessment and Reduction of Contaminant Risks

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	TAD *Branch* ETB	MD

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6 TP/USEPA/US	TAD *Branch* ETB	MD 67

SECTION A - Description of Project

1. Study objectives, presented in non-technical language such that it is understandable by non-scientific persons, including how the study addresses health protection. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Disinfection of public water supplies has been a major success in decreasing disease; however, the disinfectant reacts with materials in the source water to produce hundreds of disinfection by-products (DBPs), some of which have been associated with adverse health effects in epidemiological studies and animal toxicity studies. Although chlorination is the disinfection method used by most US water utilities, chloramination (i.e., chlorine plus

ammonia) is an alternative method that is becoming increasingly common because it forms smaller amounts of the EPA-regulated DBPs. However, chloramination produces other DBPs, many of which with little, if any, toxicological data available. A related concern is that the choice of disinfection method, along with the levels of bromine and iodine in source waters, influences the levels of brominated and iodinated DBPs in tap water. In general, iodinated and brominated DBPs are more toxic than chlorinated DBPs, but specific data on many brominated, and especially, iodinated DBPs are lacking.

Here, in order to improve the Office of Water's risk assessment of DBPs from both chlorination and chloramination, we propose to fill data gaps and examine structure-activity relationships regarding the developmental toxicity of selected chemical classes of DBPs (trihalomethanes, haloacetic acids, halonitromethanes). We plan to conduct developmental toxicity assays using F344 rats, a strain we have shown to be particularly sensitive to toxicant-induced pregnancy loss and fetal eye malformations. Briefly, this developmental toxicity screen will involve exposure of F344 rats during gestation, allowing the dams to deliver, and examination of litters for growth and physical abnormalities.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

In vivo testing is an essential part of assessing reproductive and developmental toxicity hazard in that the intact live animal is the only test system available that incorporates the complex maternal-embryonic interactions of development.

b. Justify the species requested:

Rats have been used extensively in this field because of their size, ease of care, fecundity, and large historical database. Our work will gain insights from, and add to, the large historical database of reproductive and developmental toxicity research in this species.

3. How was it determined that this study is not unnecessary duplication?

A separate PubMed search was conducted on each chemical name (listed in Section D) with the terms "developmental toxicity" and "rats." Although some of these chemicals have been tested for developmental toxicity in other strains or as part of DBP mixtures, with one exception (bromoform), none of them have been individually tested in F344 rats. (Bromoform was tested in our laboratory, in a different facility and under different conditions, over 20 years ago; we will need to re-test this chemical to provide better comparison with the current assays.) Thus, the proposed work is not unnecessary duplication.

SECTION B - In Vivo Procedures

1. Briefly describe experimental design. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

For each assay, timed-pregnant rats will be purchased and dosed by gavage on gestation days 6-15. Dams will be allowed to deliver. Dams will be monitored at term to determine gestation length (and monitor for difficulties with parturition). Litters will be examined on postnatal days 1 and 6; each pup will be sexed, weighed, and examined for abnormalities. After the day-6 examinations, dams and pups will be euthanized. At necropsy, maternal uteri will be examined to count implantation sites. Uteri of females that did not bear litters will be stained to detect cases of full-litter resorption.

For each chemical (except bromoform), we will conduct a dose-range-finding assay to determine appropriate dose levels for the full developmental toxicity assay. (For bromoform, we already have data that will be sufficient for dose setting.)

Dose-range-finding studies
We request 30 animals (6 dams per dose level) to evaluate controls plus four dose levels per chemical.
Dose levels: 0, 25, 50, 75, 100% of high dose (see Sections B5 and D1 for maximum doses).

Definitive studies
We request 96 animals; 24 dams per dose level.

Dose levels (percent high-dose): 0, 25, 50, 100% of high dose (based on results of dose-range-finding study)

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

Based on our data and experience running developmental toxicity assays, we expect that six dams per dose group, and four dose levels (plus control) will be adequate to evaluate a dose-response and identify an appropriate high dose level for subsequent studies.
6 dams/group x 5 groups x 11 chemicals (excluding bromoform, we already have dose-setting data) = 330 dams

For the definitive studies, regulatory guidelines for developmental toxicity studies recommend approximately 20 dams (i.e., with confirmed pregnancy) per treatment group. Based on historical pregnancy rates, ordering 24 timed-pregnant females per group should give us the recommended number of confirmed pregnancies.
Note: We will conduct the studies in two blocks; if results from the first block are conclusive, we will not conduct the second block.
24 dams/group x 4 groups x 12 chemicals = 1152 dams

Overall: 330 + 1152 = 1482 dams

Offspring:
F344 rats average eight pups per litter. Assuming all dams bear a litter...
1482 litters x 8 pups/litter = 11,856 pups

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	1482	11856
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. For tracking purposes, please check if this LAPR includes any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Dams will be dosed once daily by gavage on gestation days 6-15 with curved 3", 18- or 20-guage, stainless steel ball-bearing-tipped gavage needles.

Doses will be selected to avoid overt maternal toxicity. Maximum dosages are shown below.
For the dose-range finding studies, dose levels: 0, 25, 50, 75, 100% of the maximum dose.

For the definitive studies, the high dosage will be selected based on the results of the dose-range finding study.
Dose levels will be 0, 25, 50, 100% of high dose

Chloroform, max dose: 400 mg/kg
Bromoform, max dose: 150 mg/kg
Iodoform. max dose: 60 mg/kg
Dichloroacetic acid (DCA), max dose: 900 mg/kg
Dibromoacetic acid (DBA), max dose: 200 mg/kg
Diiodoacetic acid (DIA), max dose: 100 mg/kg

Trichloroacetic acid (TCA), max dose: 2000 mg/kg
Tribromoacetic acid (TBA), max dose: 100 mg/kg
Chloropicrin (trichloronitromethane, TCNM) max dose: 10 mg/kg
Bromopicrin (tribromonitromethane, TBNM), max dose: 8 mg/kg
Iodopicrin (triiodonitromethane, TINM), max dose: 6 mg/kg
Dibromonitromethane (DBNM), max dose: 10 mg/kg

For DCA, DBA, DIA, TCA, and TBA, dosing solutions will be prepared in deionized water and neutralized to pH 6-7 with sodium hydroxide. Dosing volume = 10 ml/kg.

For the remaining chemicals, dosing solutions will be prepared in corn oil. Dosing volume = 1 ml/kg.

Dams will be earmarked (holes and notches) for identification.

b. Survival Blood Collections (method, volume, frequency):
Blood will be collected (only once per animal) on GD 10 for measurement of luteinizing hormone and progesterone. Animals will be held in an acrylic restrainer for approximately 5 minutes while approximately 300 ul blood is collected from the tail vein using a butterfly needle (19G, 21G, or 23G, as appropriate for the size of the animal).

Generally, gentle stroking of the tail is sufficient to provide adequate circulation for blood collection. However, if needed (this is rare), tails will be warmed to increase circulation by dipping in warm (not hot) tap water for up to 1 minute.

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):
Litters will be examined on PND 1 and 6. Pups will be sexed, weighed, and examined for morphological and clinical abnormalities.

At necropsy of dams, uterine implantation sites will be counted.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:
For blood collection from the tail vein, animals will be held in an acrylic restrainer for approximately 5 minutes.

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

f. Describe how animals will be monitored (e.g., frequency of observations, by whom):
Animals will be monitored by laboratory staff (listed in Section E).
During treatment periods, animals will be examined after each individual animal is dosed, after all animals have been dosed, 1-4 hours post-dosing, near the end of the work day, and the next morning.
Maternal body weights will be measured daily on GD 5-16, and on GD 20.
Dams will be monitored several times per day for signs of parturition starting on GD 20.
In addition to thorough litter examinations on PND 1 and 6 (see section B5c), cageside examinations will be done daily on weekdays to check the general condition of the litter and cases of poor maternal care.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Survival Blood Collection (method, volume, frequency):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water deprivation, light cycles). Provide details regarding the length of deprivation:

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (drugs, dosages, volume, and route of administration). The use of paralytic or neuromuscular blocking agents without anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, frequency and duration of monitoring, responsible personnel, removal of sutures/staples):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animals be subject to more than one major surgical survival procedures?
◯ Yes ◯ No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. Describe actions to be taken in the event of expected or unexpected deleterious effects from procedures or chemical exposures.
Changes in body condition will be monitored. If animals (dams or pups) show symptoms of physical injury, severe toxicity, marked dehydration, dystocia, or deteriorating body condition (i.e., below "well-conditioned") we will euthanize or otherwise follow AV recommendations. For the halonitromenthanes, we will be especially attentive to signs of corrosivity (vocalization, labored breathing, excessive salivation); if such signs are noted, we will euthanize or consult AV.

b. State criteria for determining temporary or permanent removal of animals from the study.
If animals (dams or pups) show symptoms of physical injury, severe toxicity, marked dehydration, dystocia, or deteriorating body condition (i.e., below "well-conditioned") we will euthanize or otherwise follow AV recommendations. Animals with labored breathing will be removed from the study and euthanized.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 1482

b. Animals to be transferred from another LAPR:

LAPR Number that is the source of this

transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 1482

2. Species (limited to one per LAPR): Rat(s)

3. Strain: F344

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

none

4. Sources of animals:

Harlan Laboratories

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

No. *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

none

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

Because of timing constraints involved with breeding and shipping, F344 animals must arrive on GD 5; treatment will begin on GD 6.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

none

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Dams will be housed two per cage with pine shavings as bedding. Enviro-dri will be provided.

To maintain individual dam-litter identity, dams will be housed one per cage beginning GD 16-18.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.

Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Chloroform. max dose: 400 mg/kg. LD50 (rat, oral): 300-1250 mg/kg
Bromoform max dose: 150 mg/kg LD50 (rat, oral): 600-1147 mg/kg
Iodoform max dose: 60 mg/kg LD50 (rat, oral): 355 mg/kg

Dichloroacetic acid (DCA), (sodium dichloroacetate, pharmaceutical grade)
max dose: 900 mg/kg LD50 (rat, oral): 2820 mg/kg
Dibromoacetic acid (DBA) max dose: 200 mg/kg LD50 (rat, oral): 1737 mg/kg
Diiodoacetic acid (DIA) max dose: 100 mg/kg LD50 (rat, oral): no data
Trichloroacetic acid (TCA) max dose: 2000 mg/kg LD50 (rat, oral): 3320 mg/kg
Tribromoacetic acid (TBA) max dose: 100 mg/kg LD50 (rat, oral): no data

Chloropicrin (trichloronitromethane, TCNM) max dose: 10 mg/kg LD50 (rat, oral): 250 mg/kg
Bromopicrin (tribromonitromethane, TBNM) max dose: 8 mg/kg LD50 (rat, oral): no data
Iodopicrin (triiodonitromethane, TINM) max dose: 6 mg/kg LD50 (rat, oral): no data
Dibromonitromethane (DBNM) max dase: 10 mg/kg LD50 (rat, oral): no data

Corn oil (food grade, 100% pure, used within 1 year of opening) - vehicle for hydrophobic chemicals

Note: Except for DCA, none of these chemicals are available in pharmaceutical grade.

All test chemicals listed are included in HSRP #89: In vivo developmental toxicity testing.

2. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in this LAPR, and provide:

a. Information to assure that such material is pathogen-free

b. A statement regarding any safety precautions necessary for handling the material.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal	Study design; litter	~30 years experience. Proficient in cervical

	Investigator	exams, parturition exams, body weights, gavage, clinical observations, tail bleeding, cervical dislocation, Category C procedures	dislocation, including rats >200 g. Completed NHEERL-required training.
Exemption 6	Technical Staff	Litter exams, body weights, gavage, clinical observations, tail bleeding, cervical dislocation, Category C procedures	~30 years experience. Proficient in cervical dislocation, including rats >200 g. Completed NHEERL-required training.
Exemption 6	Associate Principal Investigator	Category C procedures	~30 years experience. Completed NHEERL-required training.
Exemption 6	Technical Staff	Litter exams, body weights, clinical observations, tail bleeding	~20 years experience. Completed NHEERL-required training.
Exemption 6	Technical Staff	Litter exams, body weights, clinical observations, tail bleeding	~20 years experience. Completed NHEERL-required training.
Exemption 6	Student	Litter exams, parturition exams, body weights, clinical observations, tail bleeding, Category C procedures	Completed NHEERL-required training. He will be trained/supervised by Exemption 6 and Exemption 6 Exemption 6.
RTP-NHEERL	Tech Support	Category C Procedures	EPA IACUC Trained

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Dams and litters will be euthanized within 1 day of the postnatal day 6 litter examinations.

2. Describe the euthanasia techniques:

Method(s): Cervical dislocation (dams), Decapitation (pups)

Agent(s):
Dose (mg/kg):
Volume:
Route:

Source(s) of information used to select the above agents/methods:

_ Personal Experience, 2013 AVMA Guidelines on Euthanasia
NHEERL Best Practices: Fetal and Neonatal Euthanasia

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the 2007 American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

Cervical dislocation of rats >200g: The 2013 AVMA Guidelines for the Euthanasia of Animals recommends cervical dislocation as a method of euthanasia for rats weighing <200g when performed by individuals with a demonstrated
high degree of technical proficiency. It also states that the large muscle mass in the cervical region of heavy rats makes manual cervical dislocation physically more difficult. The Guideline's 200-g weight limit is flawed for two important reasons: 1) The additional weight acquired during pregnancy or lactation has little, if any, influence on the muscle mass of the neck. (E.g., our F344 rats typically weigh 200-250 g during late pregnancy, but their nongravid weights are <180g). 2) The technique for performing cervical dislocation described by the AVMA Guidelines is appropriate for mice, but it is an inferior technique for rats. Rather than using the thumb and index finger, the preferred technique involves placing the index and middle fingers on either side of the animal's neck (from the dorsal aspect with the palm facing rostrally). Unlike the Guideline's method, this method IS appropriate for heavier animals and is NOT physically more difficult. The Principal Investigator of this project has >25 years experience performing this technique on nongravid rats weighing >350g and pregnant or lactating rats weighing >500g.

4. Describe how death is to be confirmed.

Vital organ section, Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized as above

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. The proposed research using animals does not unnecessarilty duplicate any previous experimentation.
4. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
5. The Attending Veterinarian has been consulted in regard to any planned experimentation involving

pain or distress to animals.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. Individuals from outside of EPA who are collaborating on this project, and who conduct related experimentation on EPA procured or bred animals in their respective Institutions, have the equivalent of a current IACUC approved LAPR at their respective Institutions.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	10/15/2014

Submitted: 10/15/2014

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	10/15/2014	Exemption 6	TAD	MD
		Lotus Notes Address	***Branch***	***Submitted to Branch Chief for Approval***
	by Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	ETB	10/15/2014 04:45 PM

ATTACHMENTS

17-10-001 PI resp.pdf

Actions

First Update notification sent: 09/01/2015
Second Update notification sent:
First 2nd Annual notification sent: 08/31/2016
Second 2nd Annual notification sent: 09/28/2016
1st Expiration notification sent: 09/01/2017
2nd Expiration notification sent: 10/02/2017

History Log: